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Original Research Article

Potential Inhibitory Effects on Human Platelet Aggregation and blood Coagulation of the Aerial Part of *Portulaca oleracea* L

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ABSTRACT

Portulaca oleracea L. is a vegetable plant traditionally used to treat several conditions. A wide range of bioactivities of *P. oleracea* were reported. However, its potential in the treatment and prevention of heart diseases has rarely been documented. This study reports, for the first time, the inhibitory effect on human platelet aggregation and blood coagulation of the aerial part of *P. oleracea* L. The dried aerial part of *P. oleracea* was macerated with 80% ethanol, suspended in the water and then successively fractionated with *n*-hexane, ethyl acetate. Blood samples were collected from healthy volunteers. The antiplatelet effects of extracts were evaluated using the turbidimetric method. The anticoagulant activity was assessed by measuring prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time. The results showed that the aerial part of *P. oleracea* had potent inhibitory effects on human platelet aggregation and blood coagulation. The ethyl acetate fraction showed the strongest antiplatelet effects ($p < 0.05$) regardless of agonists used. At 2 mg/mL, percentage inhibition of platelet aggregation of the ethyl acetate fraction was up to 99.5%. Moreover, both ethanol extract and ethyl acetate fraction exhibited anticoagulant activities by prolonging both APTT and PT ($p < 0.05$), while the *n*-hexane fraction prolonged APTT ($p < 0.05$), indicating possible effects on both intrinsic and extrinsic factors involved in the coagulation pathway of aerial PO. This vegetable plant could be a valuable natural source to develop supplementary products or to investigate novel antithrombotic agents for the treatment and prevention of heart diseases.

***Keywords*:** antiplatelet, anticoagulant, cardiovascular diseases, clotting time, *Portulaca oleracea*, purslane

Introduction

Cardiovascular diseases (CVDs), including coronary heart disease, cerebrovascular disease, stroke and other problems, are a group of disorders of the heart and blood vessels. They are one of the leading worldwide causes of death.[1] One of the primary reasons for CVDs is the obstruction of blood flow to the heart or brain, caused by the build-up of fatty deposits on the inner wall of the blood arteries. If the arteries leading to the heart muscle is blocked by blood clots, a heart attack will occur. If the arteries in the brain is blocked by blood clots, this will lead to a stroke.[1] Current antithrombotic agents, including antiplatelet and anticoagulant drugs, play an important role in the treatment of CVDs. However, numerous side effects such as gastrointestinal complications, hemorrhage and drug resistance are associated with their use.[2] There is an increasing interest in medicinal herbs since 30-50% of currently used pharmaceutical and nutraceutical products derives from plants. Medicinal plants serve as a potential source for the discovery of alternative therapies with improved efficacy and decreased side effects.[3] *Portulaca oleracea* L. (PO), often called common purslane, is a well-known leafy vegetable. It is widely ditributed in many countries, especially in tropical and subtropical regions. PO is known to have great nutritional values because it is rich in vitamins, β-carotene, ascorbic acid, and α-linolenic acid.[4]

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In traditional medicine, PO was used for the treatment of several conditions such as respiratory and gastrointestinal disorders, headaches, inflammations, kidney and liver diseases.[4,5] Studies have revealed that PO has a wide range of biological activities, including antioxidant, antiinflammatory, antimicrobial, anticancer, immunoregulatory, antidiabetic, antiulcerogenic, hepatoprotective, antiasthmatic, neuroprotective and hypocholesterolemic activities.[4,6-9] Especially, this plant was reported to reduce hyperglycemia and could be effectively and safely used as an adjunctive and alternative therapy for type-2 diabetes.[10,11] However, its potential in the treatment and prevention of CVDs has rarely been documented. A recent study reported the protective effect of PO on cardiovascular parameters such as malondialdehyde levels and cardiac index in rat with cardiac dysfunction caused by subclinical hyperthyroidism.[12] This study reports, for the first time, the potent inhibitory effect on human platelet aggregation and blood coagulation of the aerial part of PO collected in Vietnam. The novel findings from this study will provide scientific information about the promising functions of PO in the drug development for the treatment and prevention of CVDs.

Materials and Methods

Plant collection and identification

Portulaca oleracea L. was collected in July, 2021 in Thai Nguyen, Vietnam, identified and deposited at the Department of Life Sciences, University of Science and Technology of Hanoi, Vietnam Academy of Science and Technology, Vietnam with a voucher number of PO.A.TN01

Chemical materials

Chemicals used in this study were dimethyl sulfoxide (DMSO) and aspirin from Sigma-Aldrich, adenosine diphosphate (ADP) and collagen from Chrono-Log Corporation (USA), PT (prothrombin time),

APTT (activated partial thromboplastin time) and TT (thrombin time) reagents from Dade Behring Marburg GmbH (Marburg, Germany). In addition, ethanol (EtOH), *n*-hexane and ethyl acetate (EtOAc) were purchased from China.

Plant extraction
The aerial part was washed, dried at room temperature and then powdered. The fine powder (1.0 kg) was macerated in 80% EtOH at room temperature three times. The extract was combined, filtered and evaporated under reduced pressure to obtain the total EtOH extract (APO.Et, 90 g). Then the EtOH extract (45 g) was suspended in the water, and fractionated successively with organic solvents to obtain the *n*-hexane (APO.H, 0.81 g), EtOAc (APO.EA, 0.31 g) and water fraction (APO.W, 3.6 g). The extracts were stored at 4 - 6 °C for further use. The yield of extraction was calculated as follow: Yield (%) = $m_e/m \times 100$, where m_e is the weight of dried extracts and m is the weight of the dried plant material.

Preparation of plasma samples
The study was approved by the Ethical Committee of the Vietnam National University, Hanoi, Vietnam. Healthy volunteers, who were aged 18 - 35 years old and did not use any medications for at least 3 weeks, were recruited for the study. A complete blood count test was performed before experiments to ensure that studied volunteers had normal blood cell counts. Venous blood samples were drawn from fasting volunteers and put in 3.2 % sodium citrate tubes. In order to obtain platelet-rich plasma (PRP), centrifugation of blood samples at 500 rpm in 10 min were done. However, to obtain platelet-poor plasma (PPP), blood samples were centrifuged at 3000 rpm in 10 min.

Determination of antiplatelet aggregation activity
The total extract and fractions were dissolved and diluted in DMSO to obtain working concentrations of 20, 10, 5 mg/mL. The antiplatelet effects of PO aerial extracts were evaluated using the turbidimetric method described previously by Mustard et al.[13] In brief, mixtures of 450 µL PRP and 50 µL of the plant extracts at different concentrations were incubated at 37 °C for 3 mins. Then, ADP (10 µM) or collagen (2 µg/mL) was added to the prepared mixtures for stimulation of platelet aggregation. The curves of platelet aggregation were determined by the change of light transmission. Three platelet parameters were collected over 6 min: maximum amplitude, maximum slope (S) and area under the aggregation curve (AUC). The maximum amplitude resembles the maximum platelet aggregation. While maximum slope shows the velocity of platelet aggregation, AUC indicates the overall platelet aggregation. Ticagrelor 0.002 mg/mL was used as the positive control in the case of ADP, while aspirin 0.1 mg/mL was used as the positive control in case of collagen. DMSO 0.1% was used as the negative control. The percentage inhibition (%I) of platelet aggregation was calculated by the following formula:

$$\%I = (X - Y)/X \times 100\%$$

Where X was the maximum aggregation of the negative control, Y was the maximum aggregation of the samples.

Determination of anticoagulant activity
The anticoagulant activity of the samples was assessed following the previously described method.[14] Mixtures of 450 µL PPP and 50 µL plant extracts at different concentrations were pre-incubated at 37°C. Then, 50 µL APTT and PT reagents were incubated with 50 µL of prepared mixtures at 37°C for 5 min, for APTT and TT assay, respectively. However, for the TT assay, 10 µL TT reagent was incubated with 50 µL of the prepared mixture at 37 °C for 5 min. The APTT, PT and TT parameters were measured using Sysmex CS-2100i machine (Japan). The positive control was heparin 2 IU/mL for PT assay, and heparin 0.2 IU/mL for both APTT and TT assays. The negative control was DMSO 0.1%.

Data analysis
The experiments were performed in triplicate. Data were presented in the form of mean ± standard deviation (SD). The SPSS software version 25.0 (Armonk, NY: IBM Corp.) was utilized to perform independent sample t-test and one-way ANOVA test to evaluate the difference between groups. Pearson's correlation coefficient (r) test was applied to determine the associations between variables. The results reached statistically significant if $p < 0.05$.

Results and Discussion

The percentage yield of the extraction of the aerial part of *P. oleracea* was 9.0% for the total EtOH extract (APO.Et), 0.16% for the *n*-hexane fraction (APO.H), 0.06% for the EtOAc fraction (PO.EA) and 0.72% for the water fraction (PO.W).

Inhibitory effect of P. oleracea aerial extracts on platelet aggregation induced by ADP
It was observed that the total EtOH extract and three fractions of the aerial part of PO expressed significant inhibitory effects on platelet aggregation in a dose-dependent manner (Pearson correlation coefficient, r > 0.8 for all extracts). The total EtOH extract APO.Et and the EtOAc fraction APO.EA showed strong antiaggregatory effects. At 2 mg/mL, both APO.Et and APO.EA showed very high values of percentage inhibition (%I > 90%) when platelet aggregation was induced by ADP. In addition, the EtOAc fraction exhibited the strongest antiplatelet effect compared to the total extract and other fractions ($p < 0.05$). %I of APO.EA at 2 mg/mL was up to 99.5%, which was significantly higher than the one of the positive controls ($p < 0.05$). At 2 mg/mL, the *n*-hexane fraction APO.H also showed higher %I than the positive control ($p < 0.05$), while the water fraction APO.W showed similar %I compared to the positive control ($p > 0.05$). APO.W exhibited the lowest antiplatelet effects among extracts ($p < 0.05$) (Figure 1a).

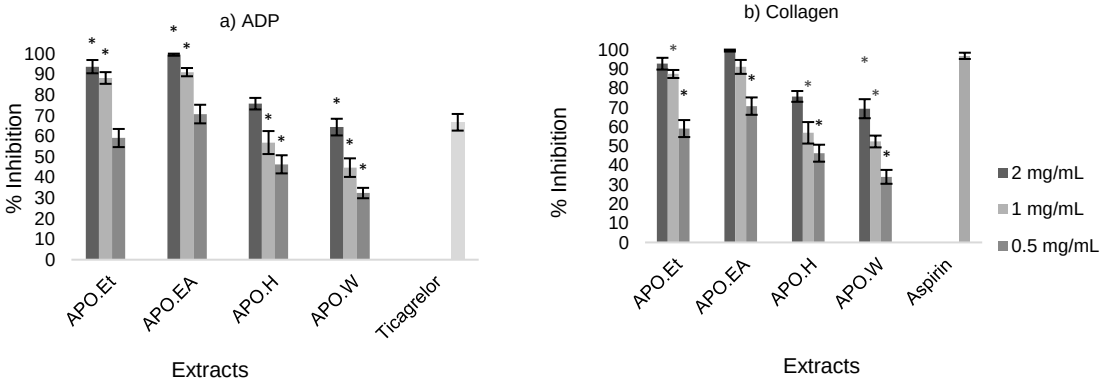

Figure 1: The percentage inhibiton of platelet aggregation induced by a) ADP and b) collagen of *P. oleracea* aerial extracts (*: $p < 0.05$ compared to the positive control; APO.Et: total EtOH extract of aerial *P. oleracea;* APO.EA: ethyl acetate fraction of aerial *P. oleracea;* APO.H: *n*-hexane fraction of aerial *P. oleracea*; APO.W: water fraction of aerial *P. oleracea*; ADP: adenosine diphosphate)

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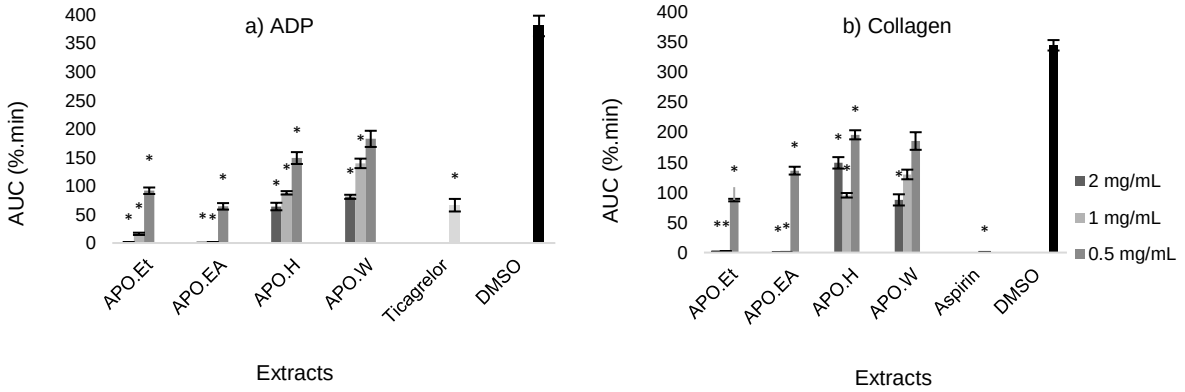

Figure 2: Area under the platelet aggregation curve induced by a) ADP and b) collagen of *P. oleracea* aerial extracts (*: $p < 0.05$ compared to the positive control; APO.Et: total EtOH extract of aerial *P. oleracea;* APO.EA: ethyl acetate fraction of aerial *P. oleracea;* APO.H: *n*-hexane fraction of aerial *P. oleracea;* APO.W: water fraction of aerial *P. oleracea;* ADP: adenosine diphosphate)

Moreover, the results showed that all extracts significantly lowered AUC and slope compared to the negative control ($p < 0.05$). These two platelet parameters were negatively correlated with the tested concentrations (Pearson correlation coefficient, r < -0.76 for all extracts). At the highest tested concentration of 2 mg/mL, both APO.Et and APO.EA showed signicantly lower slope than the positive control ($p < 0.05$). Notably, APO.EA at 2 mg/mL exhibited the lowest AUC and slope among fractions, with significantly lower AUC and slope than the positive control ($p < 0.05$). Compared to the positive control ticagrelor 0.002 mg/mL, at 2 mg/mL, the *n*-hexane fraction APO.H had significantly lower slope ($p < 0.05$) and similar AUC ($p > 0.05$), and APO.W had similar AUC and slope ($p > 0.05$) (Figure 2a, 3a).

Inhibitory effect of P. oleracea aerial extracts on platelet aggregation induced by collagen

Similar to the results obtained in the case of ADP agonist, when platelet aggregation was stimulated by collagen, the total EtOH extract and three fractions of the aerial part of PO also showed dose-dependent antiplatelet aggregation effects (Pearson correlation coefficient, r > 0.8 for all extracts). Both total EtOH extract APO.Et and EtOAc fraction APO.EA exhibited strong inhibitory activities with percentage inhibition values of > 90% at 2 mg/mL. Moreover, the EtOAc fraction had the strongest antiplatelet aggregation effect among extracts ($p < 0.05$). At 2 mg/mL, APO.EA showed extremely high %I of 99.5%, which was significantly higher than the one of the positive controls ($p < 0.05$). Both APO.H and APO.W at all tested concentrations showed antiplatelet effects, but %I was significantly lower than the postive control ($p < 0.05$). APO.W exhibited the lowest antiplatelet effects among extracts ($p < 0.05$) (Figure 1b).

Concerning AUC and slope of platelet aggregation, the results showed that these two platelet parameters of all extracts were negatively correlated with the tested concentrations (Pearson correlation coefficient, r < -0.76 for all extracts), which is also similar to the case of ADP. The total extract and three fractions significantly decreased both AUC and slope compared to the negative control ($p < 0.05$). It is noted that both APO.Et and APO.EA at the highest tested concentration of 2 mg/mL had signicantly lower slope than the positive control ($p < 0.05$). Moreover, the lowest AUC and slope among fractions were also observed for the EtOAc fraction APO.EA at 2 mg/mL. These two values of APO.EA were even significantly lower than the positive control ($p < 0.05$). However, the AUC and slope values of both *n*-hexane and water fraction were significantly higher than the ones of the positive control ($p < 0.05$). APO.W had the highest AUC and slope compared to the others ($p < 0.05$) (Figure 2b, 3b).

Anticoagulant effect of the aerial extracts of P. oleracea

The results indicated that the total EtOH extract, the EtOAc and *n*-hexane fraction of aerial PO significantly prolonged APTT compared to the negative control ($p < 0.05$). Both APO.Et and APO.EA at 8 and 12 mg/mL showed significantly higher APTT than the positive control ($p < 0.05$). The APTT of APO.H at 12 mg/mL was similar to the positive control ($p < 0.05$). Moreover, both APO.Et and APO.EA had higher PT than the negative control ($p < 0.05$). APO.EA at 12 mg/mL showed a greater prolongation of PT than heparin 2 IU ($p < 0.05$). All extracts did not have any effect on TT (Table 1).

Discussion

Platelets play an important role in wound healing, hemostasis and thrombosis. Physiologically, their primary function is to prevent and stop blood bleeding. However, in pathological processes, platelet aggregation occurs and arterial thrombi are formed, leading to the obstruction of blood flow and local ischemia. Platelet aggregation and platelet hyperactivity contribute to the development of CVDs.[15,16] Therefore, plant extracts and compounds that can inhibit platelet activation could be beneficial for CVDs treatment and prevention.[14]

This study demonstrated, for the first time, the potent antiplatelet effects of the aerial part of PO. The total extract and all fractions could suppress the platelet aggregation induced by either ADP or collagen agonist. Similar to our recent studies, [14,17,18] three important platelet parameters were analyzed to evaluate the antiaggregatory effect, including %I, maximum slope and AUC. The maximum slope indicates the maximum platelet aggregation velocity. AUC reflects the relationship between aggregation velocity and maximum aggregation, hence it shows the overall platelet aggregation.[19,20] Low AUC and slope values indicate the inhibitory effects of the sample on the overall platelet aggregation and aggregation velocity, respectively. The results proved that the aerial part of PO significantly inhibited not only the maximum aggregation, but also the aggregation velocity and the overall platelet aggregation. Moreover, the EtOAc fraction showed the strongest antiplatelet effect among fractions. The novel finding on antiplatelet effect of aerial PO would be useful for investigation of bioactive compounds from PO in further studies.

ADP and collagen are commonly used agonists to stimulate platelet aggregation. ADP activates P_2Y_1 and P_2Y_{12} receptors, while collagen activates the glycoprotein VI (GpVI), GpIa/IIa and GpIIb-IIIa receptors, granule release and the production of thromboxane A_2.[21] The potent antiplatelet activity for both ADP and collagen suggests that bioactive components present in the aerial part of PO could have different modes of actions. Active extracts and molecules of PO might stimulate P_2Y_1 and/or P_2Y_{12} receptors of ADP agonist or interfere with the thromboxane production pathway.

Moreover, this is also the first study to report the anticoagulant activity of PO. It is known that PT, APTT and TT are coagulant assays commonly used to evaluate the anticoagulant effect of plants.[14,17,18] While the APTT assay measures the clotting time in the intrinsic pathway and reflects the influence on intrinsic factors such as factors XII, XI, IX, VIII, and V, the PT test measures the clotting time in the extrinsic pathway and indicates the effects on extrinsic factors such as factors I, II, V, X and VII.[22] The EtOH extract and the EtOAc fraction of the aerial part of PO showed potent anticoagulant effects by

prolonging both APTT and PT parameters. The observed results suggest that these two extracts might act on both intrinsic and extrinsic factors of coagulation pathway. The *n*-hexane fraction had ability to prolong APTT but not PT, suggesting that this fraction might interfere with the intrinsic but not extrinsic factors of the coagulation cascade. The TT assay measures the time taken for the conversion of fibrinogen to fibrin. In the present study, PO aerial extracts did not have any effects on TT value. This indicates that tested PO extracts might not affect the process of converting fibrinogen to fibrin in the last step of the coagulation cascade.[17]

Previous studies also reported anticoagulant and antiplatelet effects of different plant extracts.[14,17,18,23,24,25,26] Similar to the present results, the EtOAc fractions of *Canna* species were proved to exhibit the most potent antiaggregatory and anticoagulant activity among fractions.[17,18] Further investigation led to the identification of antithrombotic compounds from the EtOAc fraction of *Canna* plants.[14,17,24] The extracts from leaves of *Meriandra dianthera* also expressed anticoagulant effects on APTT and PT.[23] The potential antiplatelet and anticoagulant activity of the aerial PO suggests that PO is a promissory candidate for the development of alternative therapies or supplementary products to prevent and treat CVDs.

The detected antithrombotic effect of the PO aerial part could be explained by the abundant phytochemicals present in the plant, including flavonoids, terpenoids, carotenoids, alkaloids, polysaccharides, sterols, fatty acids, proteins, vitamins, and minerals.[4] Several compounds were isolated from the aerial part of PO, in which some compounds such as kaempferol, apigenin, luteolin, genistein, quercetin, p-coumaric acid, cafeic acid and ferulic acid were reported to have antiplatelet and anticoagulant effect.[4,6,27,28] It could be seen that the aerial part of PO is a rich source of antithrombotic molecules. Further studies could investigate novel antiplatelet and anticoagulant compounds from this interesting vegetable plant.

Although PO can be nutritious and help prevent CVDs, the significant antithrombotic findings suggest that excessive consumption of this vegetable could be associated with bleeding risk. Moreover, the concomitant use of PO with anticoagulant drugs should be cautious because it can cause potentiation of conventional drugs and increase the risk of hemorrage. Nevertheless, more research should be done to investigate these possible unwanted effects and the modes of action.

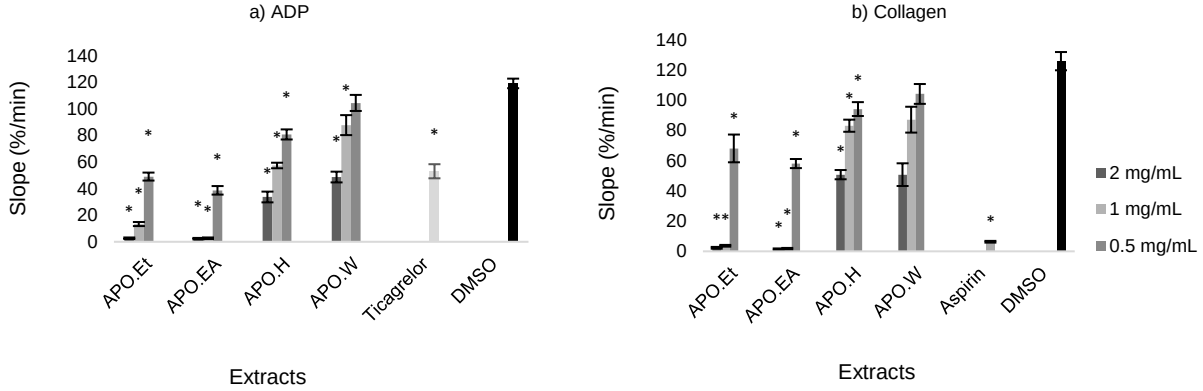

Figure 3: Slope of the platelet aggregation curve induced by a) ADP and b) collagen of *P. oleracea* aerial extracts (*: $p < 0.05$ compared to the positive control; APO.Et: total EtOH extract of aerial *P. oleracea*; APO.EA: ethyl acetate fraction of aerial *P. oleracea*; APO.H: *n*-hexane fraction of aerial *P. oleracea*; APO.W: water fraction of aerial *P. oleracea*; ADP: adenosine diphosphate)

Table 1: Anticoagulant effect of the total extract and fractions of *PO* aerial part

Sample	Conc. (mg/mL)	APTT (s)	PT (s)	TT (s)
APO.Et	12	91.40 ± 2.19*	19.97 ± 0.61*	17.2 ± 0.26
	8	54.90 ± 3.42*	12.57 ± 0.58	16.87± 0.71
	4	27.60 ± 0.78	12.50 ± 1.40	15.77± 0.25
APO.EA	12	91.43 ± 4.10*	28.77 ± 1.21*	17.47± 0.15
	8	63.60 ± 3.73*	14.90 ± 0.40	17.87 ± 0.68
	4	27.93 ± 0.55	12.60 ±0.26	17.30 ± 0.46
APO.H	12	48.00 ± 2.21*	13.67 ± 1.25	17.67 ± 0.57
	8	26.80 ± 0.61	12.57 ± 0.61	17.90 ± 0.53
	4	26.67 ± 0.32	12.07 ± 0.75	17.23 ± 0.51
APO.W	12	27.57 ± 0.95	12.83 ± 0.85	17.90 ± 0.30
	8	24.67 ± 6.30	12.33 ± 1.08	17.17 ± 0.95
	4	23.60 ± 6.50	12.13 ± 0.35	17.43 ± 0.65
DMSO	0.1%	27.83 ± 1.10	12.03 ± 0.61	17.50 ± 0.40
Heparin	0.2 IU	45.10 ± 1.14		31.70 ± 2.33
Heparin	2 IU		28.93 ± 0.91	

Data were presented as mean ± SD; APO.Et: total EtOH extract of aerial *P. oleracea*; APO.EA: ethyl acetate fraction of aerial *P. oleracea*; APO.H: *n*-hexane fraction of aerial *P. oleracea*, APO.W: water fraction of aerial *P. oleracea*; DMSO: dimethyl sulfoxide; *: $p < 0.05$ compared to the negative control.

Conclusion

This study demonstrated, for the first time, the ability of the PO aerial part to inhibit human platelet aggregation and blood coagulation. The EtOAc fraction expressed the strongest antiplatelet activity for both ADP and collagen agonists. Moreover, the PO aerial part, especially the total EtOH extract and the EtOAc fraction exhibited anticoagulant activities by prolonging APTT and PT parameters, indicating the possible effects on both intrinsic and extrinsic factors involved in the coagulation pathway. This vegetable plant could be a valuable natural source to develop supplementary products or to investigate novel antithrombotic agents for the treatment and prevention of CVDs.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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